Some nutritional requirements and the effects of four environmental factors on spore germination and growth of *Lasiodiplodia theobromae* and *Pseudocercospora timorensis*

by E.N. OKEY and V.A. ADISA*

ABSTRACT — The effects of four environmental factors : relative humidity, temperature, pH and light regimes — on spore germination and growth of *Lasiodiplodia theobromae* and *Pseudocercospora timorensis* as well as utilization of five carbon and five nitrogen sources were investigated. Optimum germination and growth occurred at 100% RH, 30°C and pH 7 for *L. theobromae* and at 100% RH, 25°C and pH 6 for *P. timorensis*. Light regimes did not produce significant effect on spore germination and growth of *L. theobromae*, but *P. timorensis* germinated better in continuous darkness. Asparagine and potassium nitrate were best utilized nitrogen sources for growth by *L. theobromae* and *P. timorensis* respectively, while glucose was found to be the best carbon source for both fungi.

RÉSUMÉ — Étude de 4 factuers environnementaux : humidité relative, température, pH et éclairement, sur la croissance et la germination des spores de *Lasiodiplodia theobromae* et *Pseudocercospora timorensis*, et utilisation de 5 sources de carbone et de 5 sources d'azote par ces champignons. Les conditions optimales pour la croissance et la germination des spores sont : 100 % RH, 30°C et pH 7 pour *L. theobromae* et 100% RH, 25°C et pH 6 pour *P. timorensis*. Tandis que l'éclairement n'a pas d'effet sur la croissance et la germination des spores de *L. theobromae*, les spores de *P. timorensis* germent mieux à l'obscurité. L'asparagine et le nitrate de potassium sont les sources d'azote les mieux utilisées respectivement par *L. theobromae* et *P. timorensis*, tandis que le glucose est la meilleure source de carbone pour les deux champignons.

KEY WORDS : nutrition, environmental factors, spore germination, growth, fungal pathogens, *Lasiodoplodia, Pseudocercospora.*

INTRODUCTION

In Nigeria, *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (= *Botryodiplodia theobromae* Pat.) has been associated with the spoilage of fruits : citrus (ADISA & FAJOLA, 1982), tomato (FAJOLA, 1978b) and foliar diseases of yams (OGUNDANA, 1972). *Pseudocercospora timorensis* (Cke.) Deighton (= *Cercospora timorensis* Cke.) was recently reported to be associated with different leaf diseases of sweet potato (ARENE & NWANKITI, 1978; OKEY, 1985).

* Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria.

SHREEMALI (1973) reported D-glucose and L-asparagine as the best carbon and nitrogen sources for *Botryodiplodia theobromae* while glucose and glutamic acid were reported as the best carbon and nitrogen sources for *Cercospora zebrina* Pass. (BERGER & HANSON, 1963). *Cercospora* s.l. leaf spot diseases of plants are commonly observed on mature leaves and rarely on young leaves. This preferential colonization of host leaf surfaces by pathogenic fungi could be related to differences in the composition of the photosynthates and other food substances in the two categories of leaves. Though detailed studies on the nature of the few reported foliar diseases of potato caused by these pathogens in Nigeria have not been investigated (ARENE & NWANKITI, 1978), this study was therefore undertaken.

A report on the studies carried out on the effects of four environmental factors on spore germination and growth of the two fungi as well as some of their nutritional requirements is therefore presented in this paper.

MATERIALS AND METHODS

The two fungi, *Pseudocercospora timorensis* (IMI 298797) and *Lasiodiplodia theobromae* (IMI 298798) used in this study are known to cause foliar diseases of mature and older leaves of *Ipomea batatas* L. in Ibadan, Nigeria (OKEY, 1985).

The effect of relative humidity (RH) on the spore germination of both pathogens was carried out at 6 levels of RH (WINSTON & BATES, 1960) : 0%, 32.5 %, 55 %, 86 % and 100 %. Observations were made every hour for 6 hours.

The effect of temperature on spore germination and growth of the fungi was investigated using purified agar (for germination for 6 hours) and nutrient broth (for growth for 7 days). Temperatures tested vary from 5 to 40°C with 5°C increments. The effect of pH on growth was studied for a period of 7 days. The pH values of 3 - 8 were obtained as described by NOLAN (1970).

The effect of light regimes on spore germination was carried out in continuous light, continuous darkness, and alternating light and darkness (FAJOLA, 1978a); incubation was for 6 hours.

In all these experiments, spore germination was assessed with the emergence of the germ tube and expressed as the percentage of spores producing germ tubes. Growth was assessed by the mycelial dry weight method.

The utilization of various carbon and nitrogen sources by the fungi was carried out using five carbon sources (glucose, sucrose, maltose, lactose and starch) and five nitrogen sources (sodium nitrate, potassium nitrate, ammonium chloride, ammonium sulphate and asparagine). For studying the effects of carbon and nitrogen source requirements on mycelial production, the basal medium was incorporated with each of the carbon and nitrogen sources used to give 0.8 g/l carbon (OSO, 1974) and 0.485g/l nitrogen (HASIJA & AGARWAL, 1978)

except for starch which was added to give 20g/l. All treatments were replicated four times.

RESULTS

Spores of *L. theobromae* and *P. timorensis* showed maximum percentage germination at 100% relative humidity. No germination occurred at 0% RH. In both fungi, germination counts increased with the increase in RH (Fig. 1). When the ANOVA test was applied, these results showed significance ($F = 3.33$ and 4.10 respectively, p. 0.05).

In both organisms, spore germination was also affected by temperature. *L. theobromae* recorded optimum germination at 30°C while *P. timorensis* germinated best at 25°C. Minimum (at 15°C) and maximum (at 40°C) germination were recorded respectively for their spores (Fig. 1). The ANOVA carried on these results showed a significant difference in the temperature treatments ($F = 2.76$ and 4.28 respectively, p. 0.01).

Optimum growth occurred in *L. theobromae* at 30°C and at pH 7. In *P. timorensis* the highest growth occurred at 25°C and at pH 6 (Fig. 2). The results obtained showed significance when the ANOVA was applied ($F = 4.3$ and 6.5 respectively, p. 0.01 for temperature and $F = 3.0$ and 4.1 respectively, p. 0.05 for pH).

The influence of light regimes had little effect on spore germination in *L. theobromae* but *P. timorensis* germinated best in continuous darkness (Table 1). The ANOVA showed that the results were not significant ($F = 18.0$, p 0.01) for *L. theobromae* but were significant for *P. timorensis* ($F = 6.9$, p. 0.05).

Table 1 – Effect of three light regimes on spore germination of *L. theobromae* and *P. timorensis* on purified agar medium for 6 hr. (data are means of four replicates, * = standard error).

Tableau 1 – Effet du régime d'éclairement sur la germination des spores de *L. theobromae* et *P. timorensis*, sur milieu agar purifié, pendant 6 h. (Les résultats sont les moyennes de 4 répétitions, * = erreur standard).

light regimes	% spore germination	
	L. theobromae	*P. timorensis*
continuous light	60.0 ± 1.1*	35.3 ± 3.1*
continuous darkness	60.0 ± 2.5	58.7 ± 1.2
alternating light/darkness	58.7 ± 3.3	43.0 ± 1.7

Glucose was found to be the best carbon source for both fungi (Fig. 3). The ANOVA showed significance ($F = 3.84$, p. 0.05 for *L. theobromae* and

Fig. 1

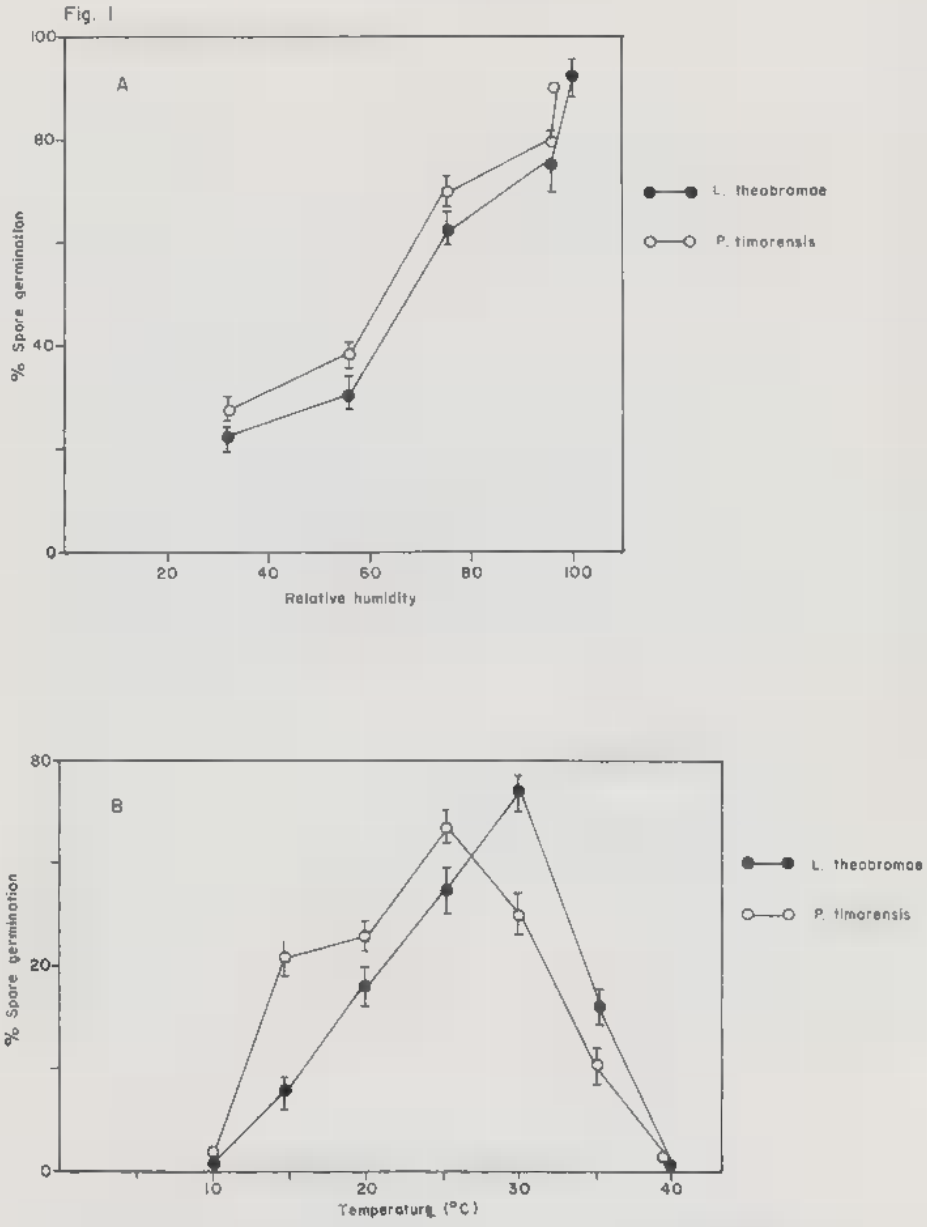

Fig. 1 — Effects of relative humidity and temperature on spore germination of *L. theobromae* and *P. timorensis* during a 6 hr incubation period on purified agar medium. Results are means of 4 replicates.

Fig. 1 — Effets de l'humidité relative et de la température sur la germination des spores de *L. theobromae* et *P. timorensis*, incubation de 6 h sur milieu agar purifié. Les résultats sont les moyennes de 4 répétitions.

Fig. 2

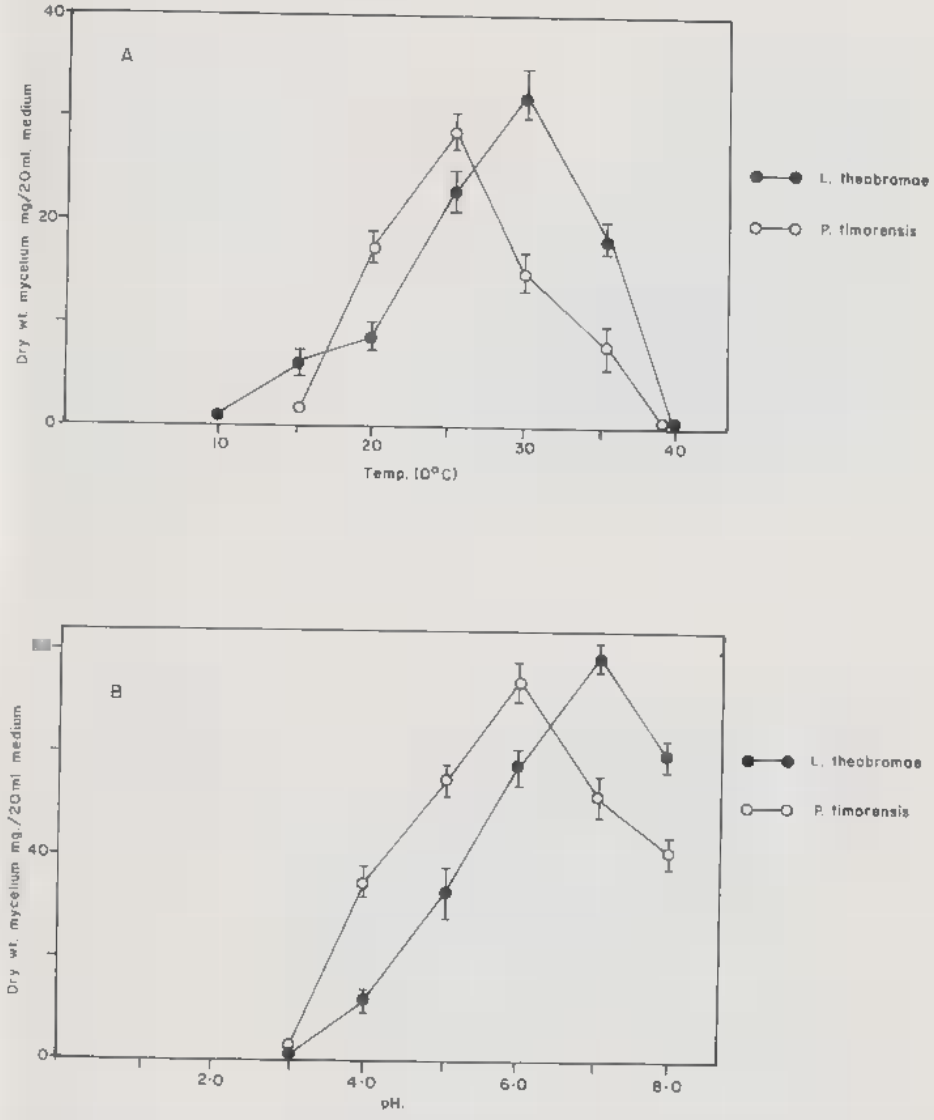

Fig. 2 — Effects of temperature and pH on the growth of *L. theobromae* and *P. timorensis* when grown in nutrient broth liquid medium for 7 days. Results are means of 4 replicates.

Fig. 2 — Effets de la température et du pH sur la croissance de *L. theobromae* et *P. timorensis*, en milieu liquide nutritif pendant 7 jours. Les résultats sont les moyennes de 4 répétitions.

F = 4.16, p. 0.05 for *P. timorensis*). *L. theobromae* best utilized asparagine while *P. timorensis* grew best in potassium nitrate. The results showed significant

Fig. 3

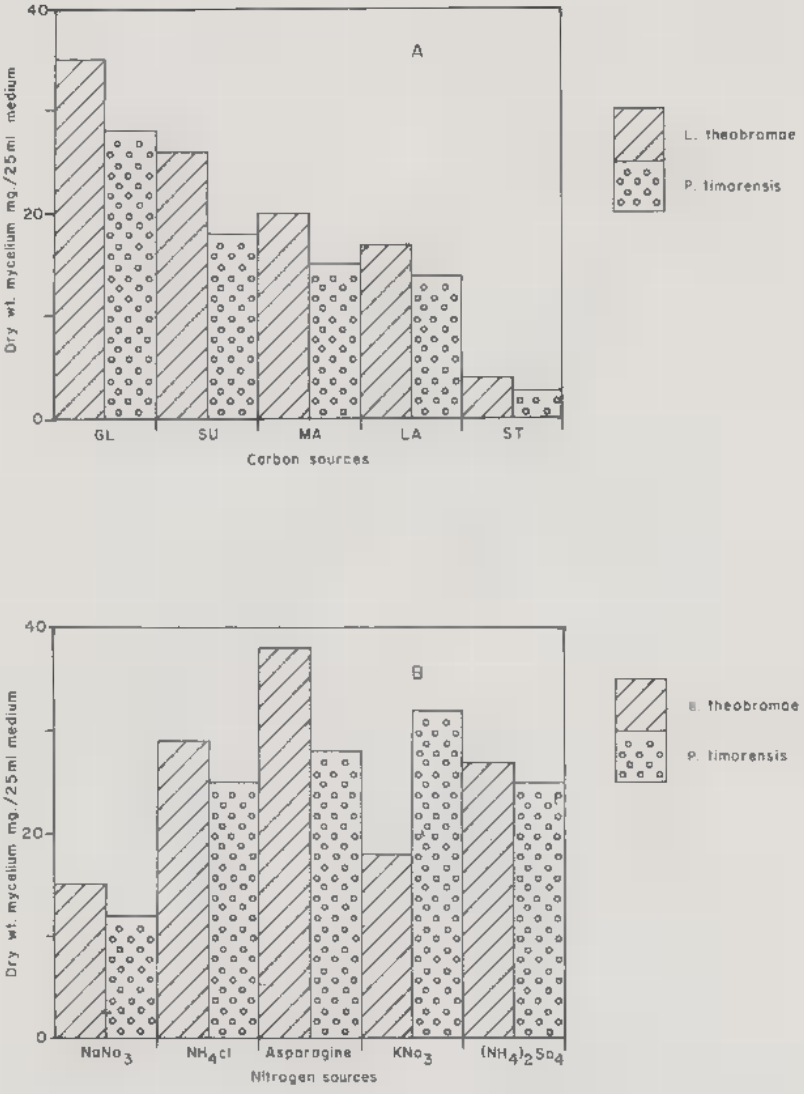

Fig. 3 – Utilization of 5 carbon and 5 nitrogen sources by *L. theobromae* and *P. timorensis* cultured in nutrient broth liquid medium (as basal medium) for 7 days. Results are means of 4 replicates.

Fig. 3 – Utilisation de 5 sources de carbone et 5 sources d'azote par *L. theobromae* et *P. timorensis* en milieu liquide nutritif (comme milieu de base), pendant 7 jours. Les résultats sont les moyennes de 4 répétitions.

differences when the ANOVA was carried out ($F = 3.8$, p. 0.01 for *L. theobromae* and $F = 4,2$, p. 0.01 for *P. timorensis*).

DISCUSSION

High relative humidity levels of 80-100 % were found to enhance spore germination in *L. theobromae* and *P. timorensis*. Moisture is a primary factor in germination because it is needed for the hydration of the highly dehydrated proteins within the spores (MARTIN & NICHOLAS, 1970). The enhancing influence of increased RH was indicated by the greater number of germinating spores and also in the rapidity of the elongation of the germ tubes. Such high moisture requirements were observed for *Botryodiplodia theobromae* by EKUNDAYO (1970) and for five *Cercospora* spp. (FAJOLA, 1978a).

Temperature also had a significant effect on the germination and growth of both fungi. The influence of heat on fungal development is reflected in almost all physiological processes primarily by changing the rate of cellular reactions (GOTTLIEB, 1950). In this study, temperature between 25-30°C was optimal while temperatures below 15°C and above 35°C were unfavourable for spore germination and growth. Similar observations were made for *Cercospora apii* Fres. (EMUA, 1980) and *Botryodiplodia theobromae* (EKUNDAYO, 1970).

The different light regimes may affect the germination of fungal spores. However, during the current study, they had little effect on spore germination rate of *L. theobromae* but the effect on *P. timorensis* spores was significant. Light intensity may therefore be more important in spore germination than light regimes.

Spore germination of *L. theobromae* and *P. timorensis* were found to be greatly enhanced by high RH while optimal temperatures for spore germination and growth for both fungi obtained *in vitro* coincide with the commonly observed field temperatures. These factors could therefore not only account for their survival in the field but also enhance disease development.

Experimental results on utilization of some carbon and nitrogen sources provided some information on the preferential assimilation of these substances by fungi. The photosynthetic leaf areas of the sweet potato which they colonize are the productive sites of some of the food substances. Glucose was best utilized by both fungi. According to TANDON & CHANDRA (1962), monosaccharides are the most easily assimilable carbohydrates. That the two fungi cause foliar diseases of mature and older leaves of sweet potato might be an indication of some nutritional and physiological deficiencies in juvenile or immature leaves.

REFERENCES

ADISA V.A. and FAJOLA A.O., 1982 — Post-harvest fruit rots of three species of citrus in South Western Nigeria. *Indian Phytopathol.* 35 : 595-603.

ARENE O.B. and NWANKITI O.A., 1978 — Sweet potato diseases in Nigeria. Pest Artic. News Summ. *PANS* 24 : 294-305.

BERGER R.D. and HANSON E.W., 1963 — Relation of environmental factors to the growth and sporulation of *Cercospora zebrina*. *Phytopathology* 53 : 286-294.

EKUNDAYO J.A., 1970 – Pycnidum production by *B. theobromae*. II Germination of Pycnidiospores. *Canad. J. Bot.* 48 : 67-70.

EMUA S.A., 1980 – Leaf spot diseases of cultivated yam *(Dioscorea* spp.) in South Western Nigeria. Ph. D. Thesis, University of Ibadan, Ibadan, Nigeria.

FAJOLA A.O., 1978a – The effect of some environmental factors on the reproductive structures of some species of *Cercospora*. *Nova Hedwigia* 29 : 922-934.

FAJOLA A.O., 1978b – Psychrophiles and the cold storage of some fruits in Nigeria. Paper presented at the Annual Conf. Nigeria Soc. Pl. Protec. (Benin City, 27/2 - 1/3/78) : 2-3.

GOTTLIEB D., 1950 – The physiology of spore germination in fungi. *Bot. Rev.* 16 : 229-250.

HASIJA S.K. and AGARWAL H.C., 1978 – Nutritional Physiology of *Trichothecium roseum*. *Mycologia* 70 : 47-60.

MARTIN J.F. and NICHOLAS Cr., 1970 – Physiology of spore germination in *Penicillium notatum* and *Trichoderma lignerum*. *Trans. Brit. Mycol. Soc.* 55 : 141-148.

NOLAN R.A., 1970 – Physiological studies on an isolate of *Saprolegnia ferax* from the larval Gut of the Blackfly *Simulium vittatum*. *Mycologia* 62 : 523-540.

OGUNDANA S.R., 1972 – *Botryodiplodia* leaf spot of yams. *Tropical Root and Tuber Crops, Newsletter* 6 : 26-28.

OKEY E.N., 1985 – Studies on two foliar diseases of sweet potato *(Ipomoea batatas* L.) in Ibadan, Oyo State. M. Sc. Dissertation, University of Ibadan, Ibadan, Nigeria.

OSO B.A., 1974 – Carbon source requirements of the thermophilic ascomycete, *Chaetomium thermophile* var. *coprophile*. *Z. Allg. Mikrobiol.* 14 : 603-610.

SHREEMALI J.L., 1973 – The effect of carbon and nitrogen sources on the growth and sporulation of six different isolates of *Botryodiplodia theobromae*. *Indian Phytopathol.* 25 : 220-224.

TANDON R.N. and CHANDRA S., 1962 – Nutrition of *Colletotrichum gloeosporioides*. *Mycopathol. Mycol. Appl.* 18 : 213-224.

WINSTON P.W. and BATES D.H., 1960 – Saturated solutions for the control of humidity in biological research. *Ecology* 41 : 232-237.